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In re application of:
Wold et al.

Serial No.: 09/351,778

Filed: July 12, 1999

For: Replication-Competent Anti-Cancer Vectors

Examiner Peter Brunovskis

Group Art Unit 1632

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DECLARATION OF PRIORITY OF INVENTION UNDER 37 C.F.R. 1.131

I, William S. M. Wold, Ph.D., Professor and Chairman, Department of Molecular Microbiology and Immunology, Saint Louis University School of Medicine, am the principle investigator and an inventor of the invention embodied in the instant patent application entitled "Replication-Competent Anti-Cancer Vectors," Serial No. 09/351,778, filed July 12, 1999.

I have been studying the adenoviral death protein (ADP), which is a major component of the instant invention, since about 1984, at which time I and others published a paper in the *Journal of Virology* (vol. 52, pp. 307-313) describing the open reading frame and the polypeptide encoded by the E3 11.6K (ADP) region of adenovirus 2. My lab has been actively studying ADP and adenoviral vectors since that time. I have published several papers on the subject preceding the initial conception of our present invention. Those papers, which were published in *Journal of Virology* (1992, vol. 66, pp. 3633-3642) and *Virology* (1992, vol. 191, pp. 743-753), describe the structure and expression pattern of ADP.

I understand that in the final office action of paper no. 16 the Examiner held that our claims to anticancer vectors, which are replication competent in neoplastic cells and which overexpress the adenoviral 11.6K protein, known herein as Adenovirus Death Protein (ADP), are anticipated by the disclosures of U.S. Patents number 6,197,293 and 6,254,862 (hereinafter the Calydon patents or vectors). It is my understanding that the Examiner considers that both of those patents disclose "recombinant vector[s] which [are] replication-restricted to neoplastic cells and which overexpress an adenoviral death protein." First of all, I would like to state for the record that our anticancer vectors are distinct from the Calydon vectors in that our vectors are competent to replicate in any neoplastic cell, whereas the Calydon vectors are restricted to either liver cells (neoplastic or normal) or prostate cells (neoplastic or normal). Therefore, I strongly assert that the Calydon patents do not anticipate the instant invention. Secondly, I hereby declare that a vector that inherently overexpresses ADP and is competent to replicate in neoplastic cells was conceived by myself on or before July 20, 1994, as exemplified in a patent application number 08/277,737, and diligently reduced to practice from July 13, 1996, which precedes the filing date of the Calydon provisional applications on March 3, 1997, until the filing of the instant application on July 22, 1999.

Conception of a vector that inherently overexpresses ADP and is competent to replicate in neoplastic cells.

(1) I hereby declare that the instant invention was conceived on or before the date of filing of our U.S. patent application number 08/277,737 entitled "Adenovirus E3-11,600 MW Protein as a Therapeutic Agent to Promote Cell Death" on July 20, 1994. That application had been unintentionally abandoned and attempts to revive the application were unsuccessful. A copy of the '737 application is included as Exhibit A. The invention that is the subject of the present

application is described in Example 1 of application '737 (pages 23 to 24), which describes a vector "that will express the 11.6K protein autonomously from a promoter with high activity ... [that] may also be deleted in the adenovirus E3 gp19K protein ... and the E3 proteins 10.4K, 14.5K, and 14.7K... The E3 6.7K gene may also be deleted...The vector may be designed such that the 11.6K protein will continue to be made in high levels under control of the E3 promoter," (page 24, lines 2-9). Furthermore, the '737 application foresees that such a vector is useful "as a therapeutic to eliminate targeted cells in human" (page 23, line 25). Thus, given that (a) the claims of the instant application are drawn to an adenoviral vector competent to replicate in neoplastic cells and overexpressing ADP, and (b) the '737 application is the first disclosure of the role of the 11.6K protein (ADP) in promoting cell lysis and describes a use for a vector that expresses ADP to eliminate targeted cells, I declare that the '737 application discloses the conception of the present invention in sufficient detail to make obvious the actual reduction to practice by a virologist of ordinary skill in the art.

(2) On July 27, 1994 I wrote a proposal to Dr. Rae Lyn Burke of Chiron entitled "Adenovirus E3-11.6K Protein as a Cell Death-Promoting Agent" in which I described the properties of ADP and its potential use as an anti-cancer therapeutic. The proposal is submitted herewith as Exhibit B). The focus of that proposal was to determine whether 11.6K would induce cell death in the absence of other adenovirus proteins and, if so, to develop vectors to deliver the gene to cells. With regard to adenovirus vectors, the proposal talks about using both replication-defective vectors and/or replication-competent vectors to deliver the ADP gene to cancer cells. On page 3 of that proposal, lines 4-7, I conceive of using ADP as a cell-killing therapeutic: "Since the 11.6K protein can promote the death of adenovirus-infected cells, it has the potential use as a therapeutic agent to kill cells, e.g. malignant cells, in humans." On page 4, line 16 through page 5, line 9, I describe the conception of both *nondefective for replication* and *defective for replication* adenoviral vectors. For example, at page 4, line 17-18, I state that "[t]he nondefective vectors generally have the E3 transcription unit deleted and replaced with the [ADP] transgene." This statement generally describes our GZ1/GZ3 class of replication competent vectors that are disclosed and claimed in the instant application. I also conceived of embodiments in which the ADP expression vectors comprise tissue-specific promoters. For example, on page 8, line 13, I state "we will construct vectors with tissue-specific promoters." Thus, I describe in the proposal to Chiron, dated July 27, 1994 the complete conception of a vector that inherently overexpresses ADP and is replication competent in neoplastic cells. Furthermore, given the state-of-the-art and the high level of skill in

the art, it would be obvious for the person of ordinary skill in the art to actually reduce our invention to practice.

Diligence toward reduction to practice of a vector that inherently overexpresses ADP and is competent to replicate in neoplastic cells.

I hereby declare that the '737 application filed on July 20, 1994, or the proposal sent to Chiron Corp. on July 27, 1994, represent the conception of the present invention, with due diligence toward the reduction to practice of the present invention commencing on July 13, 1996. I present herein a timeline of the diligent development and testing of the various embodiments of the instant invention, particularly to the genus invention as stipulated in our first claim, along with the exhibition of records that support my declaration.

Antitumor vector building

My group began experiments to build a vector that overexpresses ADP and is replication competent in neoplastic cells on July 13, 1996. As part of the first experiment to test the concept that mutations in the adenoviral E1A region would prevent the adenovirus vectors from replicating in non-cancerous cells while at the same time allowing the adenovirus vectors to replicate in neoplastic cells, we initiated the expansion of a high titer stock of the adenovirus 5 mutant strain *dl1101/1107*, which contains two small deletions in the E1A gene. Recall that this mutant strain serves as the starting strain or "backbone" of the KD series of vectors described in the patent application (see Table 1, page 22). Two separate stocks of *dl1101/1107* were confirmed on September 5, 1996 and September 17, 1996. The latter stock (designated 960717) had a very high titer (2.0×10^{11} plaque forming units per ml) and was used in subsequent experiments. It is important to note that this very high titer of the *dl1101/1107* strain is comparable to titers of wild type Ad5, which, given the fact that the *dl1101/1107* stock was grown in A549 human tumor cells, indicates that virus strains harboring mutations in the E1A gene are capable of robust replication in neoplastic cells. Well controlled experiments were performed comparing the rate of plaque formation of adenovirus strain *dl1101/1107* to the rate of plaque formation of strains *dl702* (which is wild-type at E1A and ADP) in A549 cells. Those experiments demonstrated that strain *dl1101/1107* produced plaques at a rate indistinguishable from *dl702*, thereby demonstrating replication-competence in neoplastic cells.

The next step in the formation of an adenovirus vector, which is replication-competent in neoplastic cells and overexpresses ADP, was to delete the E3 region of strain *dl1101/1107* then drop-in the ADP from Ad5. On September 18, 1996, Konstantin Doronin, one of the inventors, purified the EcoRIA fragment containing the DNA sequence encoding ADP. As part of the construction of these adenoviral vectors, various deletions and insertions were made in the backbone adenoviral E3 region by PCR. For example, in the construction of KD1 and GZ1, the sequence CCTTAATTAAA was inserted into the E3 region (see Table 1, page 22 of the instant specification of U.S. application number 09/351,778). This sequence was inserted by site directed mutagenesis using a primer called KD6, which was purchased from GIBCO BRL on September 26, 1996 (see Exhibit C, page 2). The construction of the ADP overexpression vectors proceeded diligently until the KD1 vector was successfully made and confirmed in 293 cells on February 20, 1997.

The following experiments were performed between September 26, 1996 and February 20, 1997 toward the construction of the KD1 embodiment. On October 9, 1996 the ADP gene, which was isolated on September 26, 1996, was successfully subcloned into the shuttle plasmid vector, culminating in the isolation of the KD1 shuttle plasmid vector on October 11, 1996. On December 4, 1996, we cotransfected *dl*1101/1107 DNA and the KD1 shuttle vector in 293 human embryonic kidney cells to construct the KD1 adenoviral vector by homologous recombination.

Considerable time and resources were also devoted to the development and testing of other adenovirus strains inextricably related to the instant invention. For example, the "backbone" adenovirus *dl1101/1107* was tested in WI38 human diploid fibroblasts on November 24, 1996. WI38 cells are a model for non-neoplastic cells growing in cell culture because they are diploid and they undergo senescence. The tests showed that *dl*1101/1107 barely grew in WI38 cells rendered quiescent by serum starvation, a situation that resembles normal quiescent cells in the body. Similar results were observed with non-neoplastic normal HEL299 cells around Jan 31, 1997. These experiments indicate that the mutations in the E1A gene of *dl*1101/1107 preclude efficient replication of adenovirus in quiescent normal cells. This is an important aspect of the KD1, KD2 and KD3 embodiments of our antitumor vectors.

Subsequent to the constructing the first vector that overexpresses ADP, my group and I diligently worked toward making other versions or improvements of ADP overexpressing vectors prior to systematically testing these vectors for replication competence and cytolytic activity. For

example, the construction of an antitumor vector under the control of a tissue-specific promoter was begun on October 25, 1996 when the adenovirus E4 promoter was substituted with the SPB promoter, culminating, after an initial failure due to a contamination problem, in the construction and confirmation of KD1-SPB on October 17, 1997. Other vectors that were made during this period were KD3 (begun on October 30, 1996 and confirmed on April 25, 1997) and KD2 (confirmed on April 24, 1997).

Attempts to make improved versions of ADP overexpressing vectors with a wild type adenovirus backbone, in place of the *dl*1101/1107 backbone, were initiated on March 14, 1998, culminating in the confirmation of the successful production of the GZ1 vector on May 1, 1998. The successful production of the GZ3 version of the ADP overexpressing vector was confirmed on October 14, 1998. The GZ1 vector was subsequently tested according to a plaque growth assay on July 4, 1998.

Testing of the antitumor vectors

It is important to note that the testing and expansion of each vector commenced immediately upon the development of each antitumor ADP overexpression vector. On May 5, 1997, plaque development assays were conducted with vectors KD1, KD2, and KD3 and compared to the control adenovirus strain *dl*309. (*dl309* is a well known adenovirus lab strain that lacks the genes that encode the E3 10.4K, 14.5K and 14.7K proteins and is commonly used by adenovirologists as a wild type control in many experiments.) On May 9, 1997 we demonstrated that KD3 acts faster than *dl*309 in the production of plaques, and on June 13, 1997 KD1 was shown to act faster than *dl*309. A continuous series of complex experiments, which were designed to quantitatively compare the rate of plaque formation, virus spreading and cytolysis between our various antitumor ADP overexpressing vectors in various types of neoplastic and non-neoplastic cell types, was performed from May 5, 1997 through December 16, 1998. For example, on October 23, 1998, we demonstrated that the GZ3 vector produced very large plaques in non-quiescent mammalian cell culture, relative to other ADP overexpression vector embodiments. Throughout these experiments, we demonstrated that our ADP overexpression vectors replicated preferentially in non-quiescent (especially neoplastic) cells. Additionally, we demonstrated via Western blot analysis of A549 cell extracts performed on December 9, 1997 and December 16, 1997 that ADP is in fact overexpressed by KD1, KD2 and KD3 relative to *dl*309.

The antitumor ADP overexpressing adenoviral vectors that are competent to replicate in neoplastic cells were next tested in nude mouse models. We chose nude mice, given that our cytolytic vectors were designed to work on human neoplastic cells, and not murine cells. Nude mice lack an immune system and therefore are able to support the growth of heterologous cells. Normal immune competent mice harboring murine-specific tumors is not a valid model for testing anticancer vectors designed specifically for human cells. The results of preliminary tests of the ability of the early KD1 and KD3 versions of the ADP overexpression vectors to suppress the growth of A549 tumors in nude mice, which tests were performed in collaboration with Dr. Jeffrey Whitsett at the Children's Hospital Medical Center, Division of Pulmonary Biology in Cincinnati, Ohio, were reported in a document faxed to us on January 9, 1998 (which is herewith attached as Exhibit D). Follow-up reports on A549 tumor growth were faxed to us from Dr. Whitsett on April 4, 1998 and July 9, 1998 (see Exhibit D). It is important to note that Dr. Whitsett, who was an invaluable collaborator, is not to be considered an inventor since he did not contribute to the conception of the invention or to the design of any of our ADP vectors.

On July 8, 1998, we initiated our own nude mouse/A549 tumor studies at Saint Louis University to study the effective dose range of the KD1 and KD3 viruses relative to control adenovirus strains. On that date, 10^8 PFU were administered per tumor. On August 27, 1998, experiments using 10^9 PFU/tumor were initiated. Tumor "seeding" experiments were initiated on September 29, 1998 and again on December 2, 1998. Small tumor experiments commenced on December 1, 1998. The results of those studies are presented in the examples of the instant application.

To establish the broad applicability of our antitumor ADP overexpression vectors that are competent to replicate in neoplastic cells, we began controlled tests of our vectors (KD1 and KD3) on Hep3B tumors in nude mice. The tumor Hep3B experiments were initiated on February 12, 1999 and the mice were necropsied on April 21, 1999 (see Exhibit E). To further test the effectiveness of our ADP anticancer vectors, we initiated radiation tumor experiments, which ran until January 6, 2000, which was past the filing date of the instant application.

In conclusion, I declare that the instant invention, which is drawn to an anticancer adenoviral vector that expresses ADP in amounts sufficient to cause cytolysis and is competent to replicate in neoplastic cells, was completely and entirely conceived in my mind at the time of the filing of the 08/277,737 patent application on July 20, 1994, wherein I envisioned and disclosed

that high expression of ADP (E3 11.6 K) in an adenovirus vector would function as an anticancer agent. I also declare and present testimony and evidence to the effect that the experiments directed to making an ADP overexpressing anticancer adenoviral vector competent to replicate in neoplastic cells were begun on July 13, 1996 and diligently pursued up until the time of filing of the instant application on July 12, 1999.

I further declare that all statements herein made by my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of U.S. Patent Application No. 09/351,778.

Respectfully submitted,

William S. M. Wold, Ph.D.

December 20, 2001
Date